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Influence of Soil Amendments and their Conjoint Application with Microbial Consortium in Enhancing Endosulfan Degradation and Reducing its Leaching to Groundwater

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A laboratory experiment using soil columns was conducted to study the effect of soil amendments (*e.g.*, farmyard manure, press mud compost, cereal straw, gypsum and fresh cow dung) and their conjoint use with microbial consortium (*Bacillus* sp. GS20 plus other bacterial isolates) in degradation of α- and β-endosulfan and reducing its leaching to underground water. Among all the five soil amendments used in the study, application of cereal straw and gypsum @ 5 t ha^{-1} separately decreased the maximum leaching of endosulfan, whereas in conjoint application with microbial consortium, cereal straw @ 5 t ha^{-1} was more effective in reducing leaching of endosulfan.

Keywords: Soil columns, Endosulfan, Amendments, Leaching, Microbial consortium.

INTRODUCTION

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) belongs to organochlorine cyclodiene (OCPs) group of insecticides used globally, mainly in developing countries against a wide range of sucking, chewing and boring agriculture insect pests, like lepidoptera, coleoptera, heteroptera, homoptera, thysanoptera and diptera. It is reported to be used widely on a variety of food crops such as vegetables, fruits, corn, cereals, oilseeds, tea and coffee and in non-food crops like cotton and tobacco [1]. Endosulfan is considered as one of the highly persistent insecticides after DDT and though has been banned in some states of India but is being applied in other states on several crops like vegetables, cereals *etc*. at various places in the country. Endosulfan is a mixture of two stereoisomers alpha (α) 70 % and beta (β) 30 % and both are equally toxic to aquifers [2]. Low temperatures at increasing depths of soil results in persistence of endosulfan and its metabolites for long periods of time in aquatic environment. Degradation of any pesticide is affected by several environmental conditions like temperature, humidity and microbial population in soil. Endosulfan sulfate is the main oxidative metabolite formed due to degradation in aerobic soils while another metabolite, endosulfan diol is mainly formed by chemical or biological hydrolysis of this compound in anaerobic soils by bacteria [3,4].

The WHO listed endosulfan in Category II as moderately hazardous, while the US EPA categorized it under highly hazardous pesticide [1]. Residues of endosulfan affect the fertility of agricultural soil and are toxic to soil inhabitants like earth worms and soil microbes. The compound is also known to inhibit soil microflora and essential activity like nitrogen fixation [5] in soils. The rate of percolation, sorption and breakdown of a pesticide within the soil profile determines the risk of groundwater contamination [6] and the presence of endosulfan in ground waters, confirms significant mobility of this agrochemical through the soil system [7]. The movement of pesticides from soil system to underground water strongly depends on the extent to which they are retained on the soil which in turn depends on the sorption properties of different soil [8].

Currently in Asia, India is the largest producer of dynamic pesticides and ranks 12th in world for their consumption [9]. Injudicious and indiscriminate use of pesticides in agriculture sector to enhance food production for continuously rising population has led to diffusion of pesticide residues into groundwater, which has raised a serious concern regarding lives existing in ecosystem [10]. Ground water is the main source of drinking and intake of water contaminated with pesticide residues may cause long term negative health issues in humans and other living beings.

Some studies show that the application of organic amendments on soil increases its organic matter/carbon content. The sorption, transformation and transport of many agriculture pollutants in soil are reported to be controlled by presence of organic matter [11]. It has been reported that addition of organic amendments increases pesticide sorption [12] and also results in an increase in the microbiological activity due to the availability of simple organic molecules such as sugar and amino acids [13]. Microbial degradation of endosulfan may play an important role in detoxifying the endosulfan-contaminated sites in the environment. There are a few reports on degradation of endosulfan by different groups of microorganisms. However, recent reports indicated that microbial conversion of endosulfan to endosulfan diol by hydrolytic pathway is a detoxification process whereas endosulfan sulfate was found to be a terminal degradation product [14]. The amount of endosulfan sulfate as recommended by USEPA should not exceed 62 µg/L in lakes, rivers and streams to be considered as safe.

In this investigation, we have studied the degradation of endosulfan using five soil amendments individually and their conjoint application with microbial consortium in enhancing endosulfan degradation and reducing its leaching to groundwater using in soil packed columns as sorbents.

EXPERIMENTAL

A soil column study of endosulfan, using poly(vinyl chloride) (PVC) columns of 6 cm dia. and 60 cm length was carried out under laboratory conditions. Depth wise (0-15, 15-30, 30-45, 45-60 cm) soil samples were collected from the E1 field of Crop Research Centre (CRC) of the University. The soil samples collected were dried in shade and were passed through a sieve with openings of 2 mm diameter. The standard analytical methods were used for studying the general properties like mechanical analysis, pH, electrical conductivity and organic carbon percentage of the experimental soils.

The technical grade endosulfan (98 % purity) which was a mixture of α and β isomers was obtained from Sigma-Aldrich, India and the other analytical grade chemicals used in study were procured from Himedia. HPLC grade triple distilled water used in the study was prepared using quartz distillation assembly in the laboratory.

Soil amendments were collected from local farm and the microbial consortium used in the study was grown in the laboratory in Department of Microbiology.

Microbial consortia preparation: The samples of rhizospheric and subsurface soils were taken from the agriculture fields of Udham Singh Nagar, Uttarakhand, India for the isolation of pesticide degrading microorganism for the study. Before preparation of consortium the performance of the bacteria to divide and breakdown pesticides was examined. The bacterial isolate of *Bacillus* sp. GS20, showing highest tolerance towards endosulfan was used for the preparation of consortium with other bacterial isolates like *Xanthomonas sp., Achromobater* sp. *Bacillus* sp. and *Pseudomonas* sp.

Preparation of amended soil (0-15): At usual field application rate farmyard manure, press mud compost, cereal straw and gypsum @ 5 t ha^{-1} and fresh cow dung @ 0.5 t ha^{-1} were mixed with the soil of 0-15 cm depth separately. The amendments mixed soil were stored in separate polythene bags, moistened to near field capacity moisture regime (15 % w/w) and incubated for one week at 27 °C temperature. Once the amended soil was incubated, amendment mixed soil with microbial consortium treatment was prepared by adding 50 mL of nutrient broth to the amended soil.

Packing of columns: The PVC tubes used as column were cut longitudinally into three equal parts and were rejoined by covering column joints using cellophane tape. The bottom of the last column was covered using perforated polythene to avoid seepage of soil in the leachate. In column uniform layer of glass wool of 1 cm width was placed and then above it packing of acid washed river bank sand of 6 cm was done. The column was filled with depth-wise moist soil samples slowly and gently to maintain natural bulk density and was then clamped in stands for support. Twenty two columns were prepared likewise, two were of control (non-amended), ten columns were top filled with amended soil, two each for all five amendments whereas in the remaining ten columns the top of the column was filled with the mixture of soil amendment and microbial consortium. The solution containing 2 mg endosulfan in methanol was mixed with 10 g of amended or control soil and was applied uniformly on the top of the column. The top soil of column was thoroughly soaked with distilled water and the leachate was obtained on the third day. A continuous flow of water was maintained throughout the leaching process at the rate of about 4 drops (0.2 mL) per minute from the top of the column. The leachate started coming out of the columns on the third day. The leached fractions were collected, filtered and extracted for endosulfan residues.

Extraction of endosulfan from the leachate: 5 mL of the leachate was taken in a 15 mL graduated centrifuge tube and to it 5 mL of hexane for subjected to conventional liquid–liquid partitioning. The mixture was vortexed for 5 min and 1 g $MgSO_4$ were added to it. Thereafter it was centrifuged for 5 min at 4000 rpm which led to the separation of two layers. The upper organic layer was retained, filtered through 0.2 µm PTFE disc filter and further analyzed using GC.

Extraction of endosulfan from the soil: At the end of leaching event, soil columns were separated and soil of each depth was taken from the column and spread over a clean plastic sheet under shade and mixed thoroughly. The soil sample was drawn and extracted for endosulfan residues following the simplified QuEChER's method [15]. 3 g soil was taken into a 15 mL centrifuge tube and after addition of 4 mL of hexane and 5 mL of distilled water it was vortexed for 2 min. The contents of the tube were allowed to stand for 10 min after which 3 g of anhydrous $MgSO_4$ and 2 g of NaCl were added. The mixture was vortexed for 2 min more. The contents were then centrifuged for 5 min at 3000 rpm and in the aliquot obtained 150 mg PSA (primary secondary amine) reagent and 1 g $MgSO_4$ were added. The mixture in the tube was centrifuged for 5 min more which led to the separation of the two layers. The upper organic layer was decanted off and filtered through 0.2 µm PTFE disc filter for the analysis of endosulfan by GC.

Analysis of the samples using chromatographic technique: For residue analysis gas chromatograph (GC) model Chemito (Ceres 800 plus) containing a capillary column and an electron capture detector (ECD), Carrier gas: Nitrogen at a flow rate of 30 mL/min was used. The operating temperature conditions: Injector temperature: 280 °C, Oven temperature: 270 °C and Detector temperature: 300 °C. The retention time for α- and β-endosulfan was 6 and 7 min under above conditions respectively (Fig. 1).

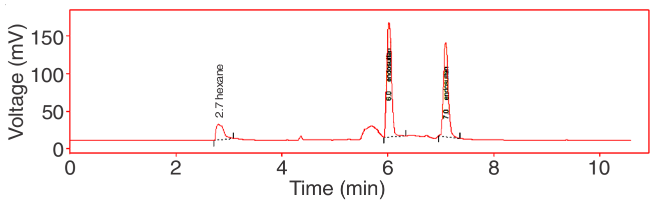

Fig. 1. Standard chromatogram of endosulfan (alpha and beta)

RESULTS AND DISCUSSION

The physio-chemical properties of coarse textured sandy loam soil used in experiment are given in Table-1. As depicted in the table, with increase in soil depth percent of sand increased while that of silt and clay decreased. Similarly the electrical conductivity and organic carbon content decreased continuously with soil depth, but pH of the soil did not vary much with increasing soil depth. Table-2 reveals the effect of different soil amendments on soil (0-15 cm) properties as compared with control. In comparison to control the pH of the soil was found to decrease by mixing of amendments in soil (0-15 cm depth) although the reduction was only minor. However the EC of the soil was enhanced by mixing of the amendments except in case of fresh cow dung where it decreased slightly. There was an increase in organic carbon content of soil by addition farmyard manure and press mud compost but the addition of other amendments did not affect the carbon content of the soil. This may be probably because both farmyard manure and press mud compost are organic manures and are initially too, rich in organic matter content.

The total amount of endosulfan (α + β) leached from the columns and that retained in soil after three days leaching event without and with microbial consortium is shown in Fig. 2. It is evident from the data that among all the five amendments, cereal straw and gypsum applied @ 5 t ha^{-1} reduced the levels of endosulfan leached, from 33.4 to 2.3 and 3.0 (µg/column) respectively during the three days leaching event. Since cereal straw is rich in organic matter content it is bound to be attracted by nonpolar organic molecules of endosulfan which increases the adsorption of endosulfan on soil and in turn prevents its leaching. Similar results have also been reported for other insecticides like chloropyrifos [16]. Though gypsum itself is not an organic amendment but definitely it adds to the value of organic amendments. High levels of soil organic matter are always associated with liberal amounts of calcium which is part of gypsum. Calcium also decreases burn out of soil organic matter when soils are cultivated by bridging the organic matter to clay [17]. However, the conjoint application of cereal straw @ 5 t ha^{-1} with *Bacillus* sp. GS20 *sp* (microbial consortium) was most effective in reducing leaching of endosulfan. This may be probably because cereal straw is rich in cellulose and lignin content and with the mixing of microbial consortia there is a significant increase in the supplementary carbon along with the pesticide which further enhances the rate of endosulfan degradation. Harish and Chauhan [18] have also reported that significant increase in bacterial cell mass enhances the rate of degradation of endosulfan. Moreover the bacterial consortium might have been able to mineralize endosulfan in both aerobic and facultative anaerobic conditions as addition of external carbon source might have enhanced the degradation efficiency. Kumar and Philip [19] have also reported the degradation of endosulfan by mixed bacterial culture in aerobic and facultative anaerobic conditions.

The effect of cereal straw as soil amendments on degradation pattern of endosulfan was also confirmed by GC-MS analysis and depicted in Table-3. Fig. 3 represents the GC-MS of control (only endosulfan (α + β) in soil) and cereal straw amended soil with endosulfan. About 98.79 % of the applied endosulfan was found to be undegraded in control whereas, it was detectable in very low percentage in cereal straw (0.72 %) amended soils. The metabolite endosulfan lactone was detected in cereal straw amended soils. Endosulfan monoaldehyde metabolite was not detectable in control but it was detected in cereal straw amended soils in 3.78 %. Similarly, endosulfan diol, endosulfan sulphate and dieldrin metabolites were not

TABLE-1
DEPTH-WISE PHYSIO-CHEMICAL PROPERTIES OF SOIL USED IN COLUMN PACKING

Soil depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH (1:2, soil water suspension)	EC (m Sm^{-1}, 1:2, soil water suspension)	Organic carbon (g kg^{-1})
0-15	64.84	20.00	15.16	7.80	0.086	6.11
15-30	70.84	18.00	11.16	8.12	0.042	5.73
30-45	76.84	14.00	9.16	7.55	0.048	4.20
45-60	80.84	10.00	9.16	8.21	0.035	3.25

TABLE-2
EFFECT ON SOME PROPERTIES OF SURFACE SOIL (0-15 cm) WITH APPLICATION OF SOIL AMENDMENTS

Soil depth (cm)	pH (1:2, soil water suspension)	EC (m Sm^{-1}, 1:2, soil water suspension)	Organic carbon (g kg^{-1})
Control	7.80	0.086	6.11
Farmyard manure @ 5 t ha^{-1}	6.77	0.131	9.63
Press mud compost @ 5 t ha^{-1}	7.54	0.351	8.65
Cereal straw @ 5 t ha^{-1}	6.74	0.158	6.29
Gypsum @ 5 t ha^{-1}	6.71	0.479	5.11
Gypsum fresh cow dung @ 0.5 t ha^{-1}	7.21	0.077	5.31

TABLE-3
DISSIPATION PRODUCTS OF ENDOSULFAN IN CONTROL AND SOILS AMENDED WITH CEREAL STRAW

S. No.	Compound name	Control		Endosulfan + cereal straw	
		Retention time (min)	Area (%)	Retention time (min)	Area (%)
1	Endosulfan lactone (-Cl)	ND	ND	13.14	0.41
2	Endosulfanmonoaldehyde (-Cl)	16.50	0.05	16.49	3.78
3	Endosulfandiol (-Cl)	ND	ND	17.38	5.61
4	Endosulfan sulphate (-2Cl)	ND	ND	19.57	4.69
5	Endosulfan alpha	19.71	57.10	19.70	0.26
	Endosulfan beta	20.79	41.69	20.46	0.46
6	Dieldrin	ND	ND	22.74	3.95

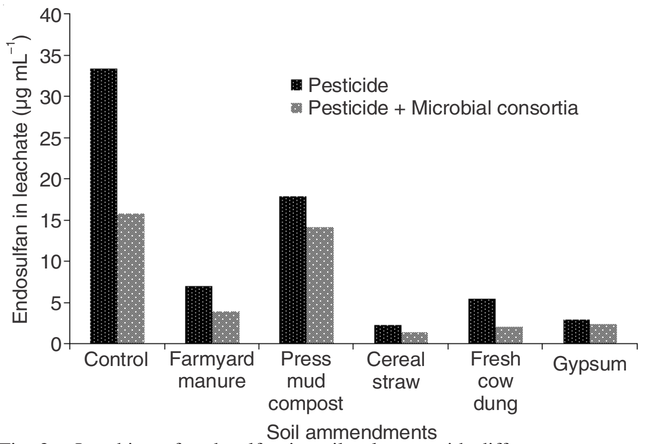

Fig. 2. Leaching of endosulfan in soil columns with different treatments along with control

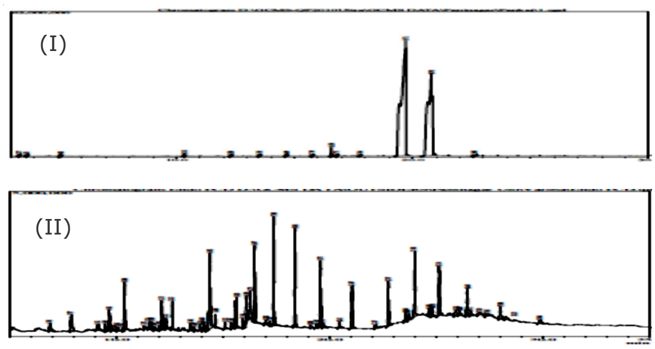

Fig. 3. GC-MS of Endosulfan in soil taken after insecticide application (I) Control (II) with cereal straw @ 5 t ha^{-1}

detectable in control but were detected in amended (both without and with microbial consortium treatment) soils. Both endosulfan diol and endosulfan sulphate formed during the degradation process get converted to non-toxic compounds efficiently within seven days [20] which are environmentally safe.

Conclusion

Based on the results it can be concluded that endosulfan is a persistent organochlorine insecticide needs to be prevented from leaching to drinking water sources and prevent pollution of water bodies. This can be achieved by mixing of soil amendment gypsum and *Bacillus* sp. consortium with the soil which will not only increase the retention of insecticide on the soil but also enhance its degradation to environmentally non-toxic components.

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